PURE LINES IN THE STUDY OF GENETICS IN LOWER ORGANISMS[1]

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AT the meeting of this society a year ago I asked in a paper read,[2] whether the pure line idea did not deserve agitating a little before this society, and I tried to agitate it. This was because I saw that for practical purposes of future work it would be necessary to make up my mind as to the importance of this idea, and it seemed that other members of the society might be in the same situation and that we might help one another. My method of agitation was to give the apparent relations of the results of work along this line up to that time, to one of the burning problems in our field—the problem of selection. In the few minutes that each of us have here the purpose of agitation can be served and general results brought sharply into view only by naked and dogmatic statements, such as one would never use under other conditions. Such naked and dogmatic presentation has serious disadvantages—felt most decidedly by the author when his critics hold the mirror up to nature. I have therefore at times regretted giving forth this paper. But if it has in any way acted as an irritant to arouse the discussion foreshadowed in our present program, I shall feel that its good results outweigh its painful ones, and that it was worth while after all. We are apparently to have brought before us a part of that "thorough try out" that I asked for, and from a study of our program I think I can see that it is not all to be a pæan of praise for the pure line work. Such illumination and such interest as comes

[1] From a symposium on "The Study of Pure Lines or Genotypes," before the American Society of Naturalists, December 29, 1910.

[2] This JOURNAL, March, 1910.

from having both sides presented I believe that we have before us.

What I wish to attempt is to give some concrete illustrations of the answer to the question discussed by Dr. Webber—What *are* genotypes? I note that some of the titles on our program speak of the genotype *hypothesis*, the pure line *theory*. What I wish to emphasize is that these things, whatever we call them, are concrete realities—realities as solid as the diverse existence of dogs, cats and horses. I find in many biologists not working in genetics an incorrigible bent for seeking under such a term as genotype something deeply hypothetical or metaphysical, and for characterizing it therefore boldly as purely imaginative. This is merely because such workers have not the things themselves before them. The genotype is merely a race or strain differing hereditarily in some manner from other races. Neither the idea nor the fact is a new one, and we should perhaps do better to discuss merely the importance of distinguishing in our work the diverse existing strains—rather than to introduce an unfamiliar term for a familiar thing. But investigation has shown the existence of these strains to play a part of such hitherto unsuspected importance that it has seemed worth while to introduce a more precise term, which shall emphasize their importance for work in genetics. In work with a certain lower organism—*Paramecium*—I have found the existence of these diverse strains or genotypes to be the guiding fact, not only for work in genetics, but for all exact work in comparative physiology. I wish to show how this is true.

We must then distinguish clearly these concrete realities called genotypes from any theories that have been built up in connection with them; from any generalizations based on their study up to this time. The existence and importance of genotypes are not bound up with any particular theory regarding selection or any other single point. In lower organisms, at least, genotypes or pure lines are merely the name for certain actual existences that you have before you; for facts that strike you

in the face. We *have,* side by side in the laboratory, a lot of diverse sets of our organisms, each set derived originally from one individual, and each differing characteristically but minutely from the others—the differences persisting from generation to generation. The behavior and properties of these things are of course a matter for further study. Can selection change them? Can environmental action permanently modify them? These are matters quite distinct from the existence of the genotypes.

To get a clear grasp of the matter, I believe that those not working with lower organisms will find it worth while to try to realize the condition which the investigator in this field has before him. A comparison may help. In lower organisms the genotype is actually isolated, each in a multitude of examples, which live along without admixture, visibly different from all others, for many generations, before again plunging into the melting pot of crossbreeding. In higher organisms we should have the same thing if every rabbit, every dog, every human being, multiplied by repeated division into two like itself, till there were whole counties inhabited by persons that were replicas of our absent president; cities made up of copies of our secretary, and states composed of duplications of the janitor I saw outside. Every human being, as it now stands, represents a different genotype (save perhaps in the case of identical twins), and these genotypes become inextricably interwoven at every generation. It is therefore easy to see how the genotype idea might appeal to workers among higher organisms as a mere hypothesis.

What then are these visible, tangible, isolated genotypes (or races, or strains) of lower organisms, and how are they distinguished? Taking *Paramecium* as a type:

1. Some of them differ in size—the size of each remaining closely constant, under given conditions, for hundreds of generations; for years. This was the first difference observed, and I tried to demonstrate it by giving measurements of successive generations of the different races. But to the worker in the laboratory these differences are evident without refined measurements; the student is at

once struck with the fact that one culture is formed of individuals that are throughout and constantly larger than those of another culture.

And here, in view of that extraordinary cry "no heredity without a correlation table"[3] (a cry that at once annihilates most Mendelian evidence of heredity), it may be well to define a little more precisely what is meant by saying that the diverse sizes are hereditary in the different races. It means that if you keep your different genotypes side by side under precisely the same conditions, you will find whenever you choose to examine and measure them, that each has a characteristic size, differing from that of the others. If therefore you follow the diverse lines from generation to generation you will get a set of chains, each with links differing characteristically throughout from the links of the other chains. It means that it is possible to predict the diverse relative sizes that will be found in the different races, and that when you examine them a hundred generations later, you will find the prediction correct. These striking facts *are what are meant* by the statement that the diverse sizes are hereditary in the different lines—and the way to determine whether the statement is true or not is to examine the lines from generation to generation to see if the statement is verified. To neglect this obvious fact; to mix all your lines together and then, in order to find out if size is inherited, to laboriously work out coefficients of correlation by refined biometrical methods—is like cutting serial sections ten microns thick of an eel, in order to find out whether it has an alimentary canal. Persons have been known to so bedevil material with refined histological methods as to quite miss the alimentary canal of an eel. The way to see it is to open the animal up and take a look at it. The way to see diverse genotypes is to isolate them and look at them and measure them and compare them. If the use of correlation tables should succeed in obscuring these striking facts (as should not be the case with proper handling) this would merely show the worthless-

[3] Compare Pearson, *Biometrika*, 1910, Vol. 7, p. 372.

ness of this method of attempting to learn the important biological facts under consideration.

2. Some of the genotypes show slight but constant differences in structure, which I shall not dwell upon here.[4]

3. They show most varied differences in their physiological characters. These physiological differences may go with differences in form and structure, or apparently they may not—so that we find types that differ, so far as detectable, *only* in physiological peculiarities.

This fact becomes of great practical importance for all physiological investigations, as a few examples from *Paramecium* will show:

(*a*) The races or genotypes differ in the conditions, both external and internal, that induce conjugation. A worker, using a certain strain, works out the conditions inducing conjugation and gives precise directions for accomplishing this. His colleague, with another strain, finds this work all wrong, and the controversy on this ancient question continues. One of my strains can be absolutely depended on to conjugate monthly if certain definite conditions are furnished; another under the same conditions never conjugates; others show intermediate conditions. These differences require no biometric methods for their demonstration.

(*b*) Again, the genotypes differ in rate of multiplication; under the same conditions some divide once in twelve hours; others once in twenty-four or more hours; others have intermediate periods.

(*c*) The genotypes differ as to the conditions required for their existence and increase. Several strains, outwardly alike, living in the same medium, are cultivated side by side on slides, in the usual hay infusion. One flourishes indefinitely. Another multiplies for ten generations, then dies out completely, and this is repeated invariably, no matter how many times we start anew our

[4] For a detailed, illustrated account of the characters, both structural and physiological, of these races, see Jennings and Hargitt, "Characteristics of the Diverse Races of *Paramecium*," *Journal of Morphology*, December, 1910.

cultures of this genotype. A third lives along in a sickly way, barely maintaining its existence.

Thus we get in our laboratory striking cases of natural selection between genotypes. To recall our comparison with human beings, if we could mix an entire community composed homogeneously of, let us say, Roosevelts, with another of copies of your ash man—which would be likely to survive? If we place together in the same culture two genotypes of *Paramecium,* as I have many times done, almost invariably one flourishes while the other dies out. This ruins many a carefully planned experiment; it must take place on a tremendous scale in nature.

What distinguishes the different genotypes then is, mainly, *a different method of responding to the environment.* And this is a type of what heredity is; an organism's heredity is its method of responding to the environmental conditions. Under a given environment the genotype *A* is large, while the genotype *B* is small. Under a given environment the strain *C* conjugates, while *D* does not. Under a given environment the strain *E* divides rapidly, *F* slowly or not at all. The various strains thus differ hereditarily in these respects, and we may say that the differences are matters of heredity. And yet we can get these same contrasts within any genotype (as our diagram illustrates), by varying the environment. The genotype *A* under one environment is large; under another it is small. Under one environment the type *C* conjugates; under another it does not. Under one environment *E* divides rapidly; under another, slowly. Are then size, conjugation and rate of fission after all determined by heredity or by environment? Such a question, when thus put in general terms, is everywhere an idle and unanswerable one. All environmental effects are matters of heredity when we compare types differing in their reaction to the environment; all hereditary characters are matters of environmental action when we compare individuals of the same heredity under effectively different environmental conditions.

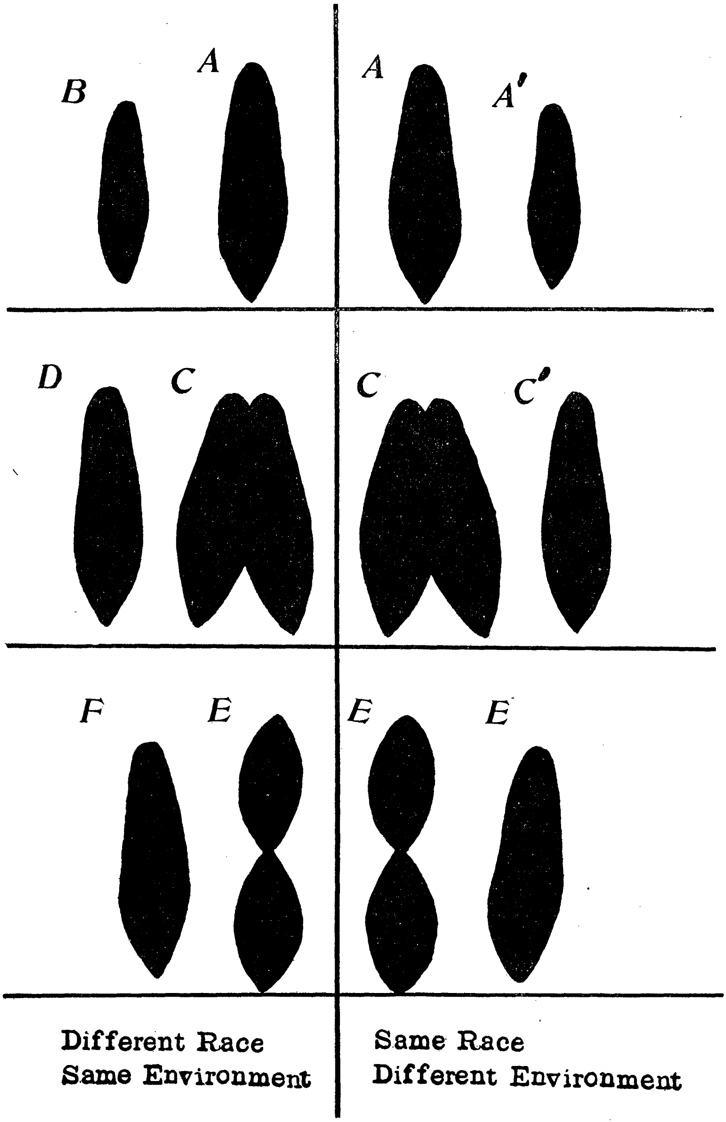

DIAGRAM TO ILLUSTRATE THE RELATION OF HEREDITY TO ENVIRONMENTAL ACTION IN DETERMINING CHARACTERS. (See text.)

Heredity has a meaning only when we (explicitly or implicitly) compare two concrete cases; when we say: To what is due the *difference* between these two cases? Otherwise we can demonstrate either that all character-

istics are hereditary (as we heard maintained at Woods Hole some summers ago); or, with Brooks, that there is no such thing as heredity. If we always compare two concrete cases, asking to what is due the difference between them, and remembering that a difference in heredity means different response to the same environment, we shall avoid these confusions, and shall find the concept of heredity most useful.

Do hereditary differentiations ever arise within our genotypes, so that from one genotype we get two? In other words, do we get from a single type strains that differ in their behavior under the same environment—the differences persisting from generation to generation? This is of course one of the fundamental questions. The genotypes of *Paramecium*, like those of most other organisms that have been carefully studied, are singularly resistant, remaining quite constant in most respects, so far as has been determined. This is an example of what gives the genotype concept its practical and theoretical importance. This is what is meant by saying that selection and environmental action are usually without inherited effect within the genotype. To find differentiations within the genotypes of *Paramecium*, we must examine certain characteristics that are most delicately poised in their responses to all sorts of conditions; such is the rate of multiplication. Studying carefully this most sensitive character, we find that differences do arise within the genotype. Under given conditions, certain rare individuals are found that divide more slowly than usual, others more rapidly, and these differences are perpetuated from generation to generation indefinitely. How are these hereditary differentiations produced?

The origin of these differentiations is in *Paramecium* as elusive as in most other cases where they have been discovered. Apparently they arise in our organism as a result of conjugation within the genotype. Certainly if after an epidemic of conjugation within the genotype we cultivate many isolated exconjugants, we find a certain small number of strains that differ in their rate of fission

from that which is typical. But the experimental analysis of this matter is still in progress, and conclusions can not yet be drawn.

It is only in rate of multiplication that I have thus far found hereditary differences arising within the pure line, and these but rarely. But this encourages one to hope that the same may be found for other characters when these are extensively studied with sufficient minuteness. The negative results thus far reached do not (as many critics have pointed out) exclude the possibility that rare cases of hereditary variation within the pure line will yet be found. What the negative results have demonstrated is that a very large share of the observed variations in organisms are not hereditary, and that selection based on these variations leads to no result—a conclusion of such great importance as to make the pure line work epoch-marking in character.

Finally, what happens when diverse genotypes mix in conjugation? To my disappointment, I have found this much more difficult to determine for the infusorian than I expected. This is owing to the fact that the conditions for conjugation differ in the diverse genotypes, so that it is almost impossible to get them to conjugate at the same time. Further, in the rare cases where two are conjugating at once, the assortative mating discovered by Pearl results in the two sets remaining separate. Thus I have not yet been able to get crosses between two genotypes whose characteristics are known beforehand; and this will be necessary before a study of inheritance, exact in the modern physiological sense, can be made. On the other hand, it *is* possible to get conjugations in wild populations that include many genotypes, and to compare the results with conjugations where but a single genotype is involved. Certain most interesting results appear. In these conjugations of mixed populations, a great number of diverse combinations are produced; the variability increases greatly, in size and in other respects. Numbers of the strains produced die, or multiply so slowly that they have no chance in competition with

those that are strong and multiply rapidly. Thus many of the combinations produced are canceled; only the strongest combinations survive. We have then on a most extensive scale an operation in natural selection and the survival of the fittest; the production of many combinations, some of which survive, while others fail. As already set forth, there is some indication of the same process in the case of conjugation within the genotype.

At our last meeting I tried to summarize the facts as to the relation of genotypic investigation to selection; it turned out that much which had been deemed a progressive action of selection was not such; and up to that time the action of selection in modifying genotypes had not been demonstrated. Similarly, I had earlier summarized the facts regarding selection in behavior, showing that it there plays a large part. I have hence suffered the peculiar fate of being belabored as an anti-selectionist in genetics, while subjected in the field of behavior to rough treatment as the champion of selection. What I tried to do in both cases was, to determine how far we had actually *seen* the effectiveness of selection—holding this question quite apart from what we believe *must* occur, or believe will be found to occur when we have seen it. It appeared clear, and still appears clear, that a very large share of the apparent progressive action of selection has really consisted in the sorting over of preexisting types, so that it has by no means the theoretical significance that had been given to it. When operating on a single isolated type it appeared that the progressive action of selection had not been seen. These are facts of capital importance to the experimenter; besides their theoretical significance, they open to each of us the opportunity to direct our efforts upon precisely this point, and so perhaps to be the first to see examples of this fundamental process not yet seen. I hoped to accomplish this myself, but after strenuous, long-continued, and hopeful efforts, I have not yet succeeded in seeing selection effective in producing a new genotype. This failure to discover selection resulting in progress came to me as a

painful surprise, for like Pearson I find it impossible to construct for myself a "philosophical scheme of evolution" without the results of selection and I would like to see what I believe must occur. It is therefore with some pleasure that I am able to record for *Paramecium* this extensive operation of selection among the diverse existing lines, and particularly in this extensive production of new combinations at conjugation, with cancellation of many of the combinations. It would seem that the diverse genotypes must have arisen from one, in some way, and when we find out how this happens, then such selection between genotypes will be all the selection that we require for our evolutionary progress. What I hope, therefore, is that some one on our program, more fortunate than myself, will be able to record seeing the actual production of two genotypes from one, or the transformation of one into another, by selection, or in any way whatever.

Yet even if this is done, we shall make the greatest possible mistake if we therefore conclude that the existence of genotypes is unimportant, and throw the matter aside; for work with a mixture of unknown genotypes will always give confused and ambiguous results, whose significance no one can know. If on the other hand we work with single genotypes, or with known combinations of them, we shall understand what our results mean. And this applies to work in other fields of biology as well as to genetics.